In Vitro Preliminary Phytochemical screening, Acute Toxicity Test and Anti-diarrheal activity of Methanolic *Bixa Orellana* Seed Extract by Castor oil induced and Magnesium Sulfate induced diarrhea models

Abstract:

Introduction: One in nine child deaths worldwide are attributable to diarrheal infections, making diarrhea the second largest cause of death among kids younger than the age of 5. Sub-Saharan Africa and South Asia have the highest mortality due to diarrheal diseases. Natural substances of mineral, animal, or plant origins have long been used in medicines, cosmetics, and food goods. *Bixa orellana* is a Brazil-native plant that also thrives in other parts of South and Central America. It is cultivated in tropical regions, including Peru, Mexico, Ecuador, Indonesia, India, Kenya, and East Africa. Due to the ban on the use of synthetic dyes in food and cosmetics, its use has increased, as it is one of the few authorized by the World Health Organization (WHO) because, in addition to being nontoxic, it does not appear to alter the nutritional content of food.Additionally, 70% of the world's natural coloring additives are sourced from annatto, a common name for *Bixa orellana*.The leaves and seed extract has been used traditionally in the tropical areas of South Asia to reduce the severity of diarrheal episodes. Therefore, this study was designed to evaluate the anti-diarrheal property of Bixa Orellana seed extracts at different doses comparing with established manufactured drugs corroborating its' orthodox therapeutic use as an anti-diarrheal agent.

Aim and Objectives: Pharmacological Evaluation of Anti-diarrheal activities in Swiss Albino Mice.

Materials and Methods:The methanolic extract of Bixa Orellana seeds was prepared by traditional method and was used to assess its' anti-diarrheal property. Anti-diarrheal activity was evaluated in Swiss Albino Mice using Castor oil induced diarrhea and MgSO4 induced diarrhea models respectively.

Observations and Results:*Bixa orellana* seed extract showed significant 75% Anti-diarrheal activity in the present study when compared to the standard drug Loperamide.

Conclusion:The methanolic seed extract of Bixa Orellana showed significant Anti-diarrheal activity therefore it ensures safe and efficient traditional uses of Bixa Orellana in times of Diarrhea.

Keywords: Bixa Orellana, Anti-diarrheal activity, Castor oil induced diarrhea,MgSo4 induced diarrhea.

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1. Introduction:

Since the dawn of human civilization, the significance of medicinal plants as a treatment modality has been underscored by their therapeutic properties. Nature has been the primary source of medicinal medicines since the dawn of time, and numerous modern active medication compounds have been identified from natural sources.[1] Medicinal plants are at priority choice because of their little or no side effects as well as reliability. Developing countries having limited resources for healthcare facility are using diversified traditional plants for healing purposes. According to WHO, the number of countries adopting national policies on traditional medicines are growing increasingly and about 80% of people are dependent on Traditional medicine worldwide. Around 21 thousand of plants are potential candidate for use as medicinal plants. [2][3]. Additionally, over 270,000 plant species have been found by humans, but it's likely that as ,many as 400,000 live on Earth.[4] This implies the need of advance research in discovering the newer effects of different plants that nature have.

The Bixaceae are a family of dicotyledonous plants which is known as achiote family. This is a small and strictly neotropical plant family comprising only one genus, Bixa, under which there are just five species namely: Bixa arborea,Bixa excelsa, Bixa Orellana L., Bixa platycarpa, Bixa urucurana [5] From all the species Bixa Orellana L. is the one which has high therapeutic value. This plant is native to Brazil, but it is found both cultivated and wild in Central America, northern South America and the West Indies. [6] It is also found in different countries with tropical weather such as India, Peru, Mexico, Ecuador, Indonesia, Kenya, and East Africa.[7]

Bixa orellana is a shrub to small evergreen tree 6–10 m (20–33 ft) high and the trunk up to 10 cm in diameter.[8] Annatto is another name of Bixa orellana, which is extensively used for coloring purposes. Bixin, a carotenoid pigment, present in the seeds of Bixa orellana, is responsible for its reddish orange color. This dye is widely used in foods, cosmetics, paints, textiles etc. This dye, being harmless, is one of the few approved dyes permitted by WHO and it does not change the food value also. [9]

Leaves, roots, seed are the parts of the plant widely used for different purposes. This plant has traditionally been used for a variety of ailments and conditions e.g., to treat diabetes mellitus [10][11], jaundice, and hypertension [12], antivenin for snakebites [13] viable chemo-preventive

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strategy for Brazilians [14] etc. Various studies reported that it has antioxidant properties [15], antimicrobial actions [16], anti-inflammatory effects [17].

Although there are many reports about different therapeutic activities of Bixa orellana, a few studies were conducted to evaluate the anti-diarrheal activity of the seeds of the plant. Our recent study was directed to investigate the anti-diarrheal effects of this particular plant.

2. Materials and Methods

2.1 Collection and identification of plant

For the tests, the fresh Bixa orellana seeds were collected in the months of September and October 2016 from Mahakhali Kacha Bazar, Dhaka. The Bangladesh National Herbarium in Dhaka verified the identity of the fresh seeds of Bixa orellana by comparing them to a voucher specimen that had been deposited there and had a different accession number, which is shown here in parenthesis: Bixa Orellana (43471). The plants were identified by the taxonomist of the Bangladesh National Herbarium (Accession No. 37848), where a voucher specimen has been stored for future access.

2.2 Extraction of Plant materials:

The separated seedswas dried in the sun for two days. It was then dried in an oven at a lower temperature (55°C) to make it appropriate for the grinding procedure. With intermittent stirring, powdered materials were steeped in methanol for seven days. The liquid supernatant was then decanted and put through a cotton plug filter. The procedure was repeated till the extraction was finished. The extract was subsequently dried, and the experiments utilized this crude extract.

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2.3 Extraction procedure:

In a conical flask, 900ml of 95% methanol are added to 150g of coarse powder, which was then blocked with cotton, covered with aluminum foil, and stirred continuously for 20 days. After twenty days, the mixture was filtered and the filtrate was collected for extract preparation. The filtrate was evaporated with a rotary evaporator and exposed to normal air for a few days so that any leftover solvent would evaporate. Finally, the residue was collected and weighed (24.4gm). The residue was kept in an airtight container.

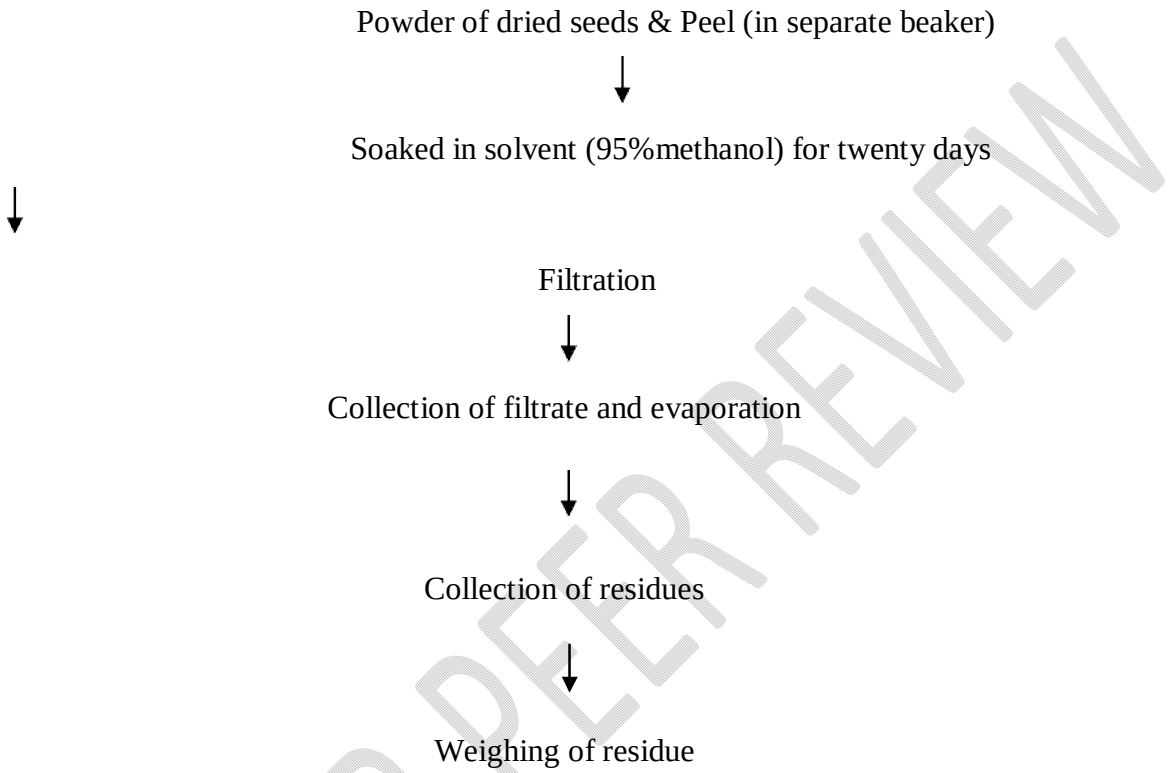

Chart 1: Preparing steps of *Bixa orellana* seeds extract.

2.4 Phytochemical Screening Test:

Using conventional procedures, the hydromethanolic seed extract of Bixa orellana was examined to identify the presence of active phytochemical components. "Thereby, analysis was conducted for alkaloids, saponins, flavonoids, terpenoids, phenols, steroids, glycosides, and tannins".[18]

2.5 Acute Toxicity test

According to the OECD 425 (2008) criteria, an acute toxicity test had been performed.Six non-pregnant adult female Swiss albino mice weighing 25–30 g were arbitrarily grouped and housed in a plastic cage.After a three-hour fast, a single female

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mouse was administered a single dose of 2,000 mg/kg of the crude extract via oral ingestion.The mouse was then closely monitored every 30 minutes for four hours for indicators of toxicity and mortality over a period of twenty-four hours.Based on the results of the initial animal, the remaining five female mice were fasted for three hours before receiving a single administration of the test extract sequentially.The animals were then monitored for 14 days for indicators of toxicity and mortality.

2.6 Anti diarrhoeal activity test in castor oil and magnesium sulfate induced diarrhea:

The antidiarrheal activity of an methanolic seed extract of Bixa orellana was evaluated by minimal modification of the method.[19]

2.7 Drugs and Chemicals:

1. Castor oil:Universal Packaging (packed in spain).
2. Loperamide Hydrocloride(Imotil,square pharmaceuticals).
3. ORSaline-N(SMC Enterprise Ltd).
4. MgSO4 (packed in China).

2.8Materials:

1. Syringe
2. Pipette
3. Feeding needle
4. Morter & pestle
5. Beaker
6. Voluemetric flask

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SI No.	Equipment	SI No.	Equipment
1	Conical flask	10	Aluminium foil
2	Rotary evaporator	11	Test tube
3	Glass rod	12	Test tube holder
4	Filter paper	13	Water bath
5	Beaker	14	Dropper
6	Funnel	15	Pipette
7	Balance	16	Pipette filler
8	Measuring cylinder	17	Spatula

Table 1: Equipment used for Extraction and antidiarrheal screening**:**

2.9 Animals:

Swiss albino mice (weighing between 22 and 25 g) were purchased from the Bangladesh-based International Center for Diarrheal Disease and Research's Animal Resources Department (ICDDR, B).The animals were kept in a constant room environment of 25.0–2.0°C, 55–65% humidity, and a 12h light–12h dark cycle.The animals were given a regular diet (ICDDR, B formulated) and were given unlimited access to water.

2.10 Making of groups of animals:

All of the test animals were initially tested with 0.5 ml of castor oil, and those exhibiting diarrhea were chosen for the experiment.On the basis of the various extract doses, the animals were split into four groups: a control group, a positive control group, and two test groups using a methanolic extract of *Bixa orellana*.Five mice were picked from each group, and they were then caged.

2.11 Antidiarrheal activity test in castor oil-induced diarrhea :

The vehicle (normal saline) was administered orally to the control group at the start of the experiment in a dose of 10 ml/kg. Loperamide was given orally to the positive control group at a dose of 3 mg/kg. The test groups were given oral doses of 250 and 500 mg/kg body weight of Bixa orellana rind extract. Then each animal was put into its own cage. Blotting paper was used to line the cage's walls and floor. Every hour, the floor lining was changed. After the aforementioned treatments, each mouse received 0.5 ml of castor oil orally to produce diarrhea.

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The total frequncy of defecation that the animals excreted during the course of a 4-hour observation period were counted and the percentage of diarrhea inhibition was calculated. [20]

2.12 Antidiarrheal activity test in magnesium sulfate induced diarrhoea :

Magnesium sulfate-induced diarrhoea was introduced similarly to castor oil.Diarrhea was caused after oral administration of magnesium sulfate at 2 g/kg to the animals 30 min after pre-treatment with vehicle (normal saline, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, and the methanol extract of Bixa Orellana at 250 and 500 mg/kg to the test groups caused diarrea. Alladministrations were oral. The observation followed the castor oil-induced diarrheal activity test protocol. [21]

2.13 Data Quality Control

The data quality was ensured by grouping experimental animals using a simple random sample strategy, blinding the system for data collection of all parameters, retaining and implementing standard procedures, and utilizing analytically graded materials.

2.14Statistical Analysis

The experimental outcomes were presented as mean standard error of the mean (SEM), and statistical significance was determined using one-way analysis of variance (ANOVA),Dunnet test.The findings are of statistical significance when the value is less than 0.05.

3.Result and discussion:

3.1 Extraction yields of Plant material:

It was determined that out of 150 gof coarse powder of *Bixa Orellana* seeds, a total of 24.4 g of crude extract was obtained, for a percentage yield of 16.26% (w/w).

3.2 Phytochemical Screening test:

The hydromethanolic extract of Bixa Orellana demonstrated the presence of flavonoids, tannins, terpenes, coumarins, and saponins upon phytochemical analysis. Nevertheless, no alkaloids or anthraquinones were detected in the sample (Table 2).

Classification of compound	Reaction
Alkaloids	-
Anthraquinones	-
Tarpenes	+
Tannins	+
Flavonoids	+

Saponins	+
Coumarins	+
Polysaccharides	+
** +: Present, -: Absent	

Table 2: Preliminary Phytochemical screening of hydeomethanolic extract of Bixa orellna seeds

3.3 Acute oral toxicity

Following oral administration of a single dose of 2000 mg/kg of the hydromethanolic extract of the seeds of *Bixa orellana*, no explicit toxicity or mortality was seen over the 14-day follow-up period.In addition, no indications and symptoms of toxicity, such as behavioral, neurological, autonomic, or physical abnormalities, were seen in the toxicology research.

Normal fluid absorption in intestine is reduced in diarrhea. Castor oil is an agent which causes diarrhea. Recinoleic acid of castor oil stimulates peristalsis as well as causes increased renal tubular secretion. Thus, it causes diarrhea.[22] In this trial, the extract (250mg/kg and 500 mg/kg) reduced the number of fecal droppings, water content in feces as well as percentage of defaecation in castor oil induced diarrheal mice. When compared to the standard drug,the extract at 500 mg/kg provided similar anti-diarrheal activity, which validates its' traditional use to reduce the symptoms of diarrhea.

Table 3: % Inhibition of defaecation of *Bixa orellana* extract as well as Loperamide in Castor oil induced method

Treatment	Dose (P.O.)	No of droppings in 4 hrs	% Inhibition of defaecation
Castor oil (Control)	0.5 ml/mouse	4±0.4082	-
Loperamide	3 mg/kg	$1\pm0.4082^{a**}$	75%
Bixa orellana extract	250 mg/kg	$1.5\pm0.2887^{a**}$	62.5%
Bixa orellana extract	500 mg/kg	$1\pm0.4082^{a**}$	75%

The values were analyzed by One way ANOVA, Dunnett test: Comparing all vs. control. Values were represented as Mean± S.E.M. (n=4). The results were considered as statistically significant, more significant and extremely significant when $p<0.05$ *, $p< 0.01$**, $p<0.001$***. a=compared with Castor oil control group.

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On the other hand, magnesium sulfate also induces diarrhea by the prevention of sodium chloride and water reabsorption and increasing the volume of intestinal content. Released cholecystokinin from duodenal mucosa results diarrhea. [23] The extract (250mg/kg and 500 mg/kg) also reduced the number of fecal droppings, water content in feces as well as percentage of defaecation in magnesium sulfate induced diarrheal mice.

Table 4: % Inhibition of defaecation of *Bixa orellana* extract as well as Loperamide in $MgSO_4$ induced method

Treatment	Dose (P.O.)	No of droppings in 4 hrs	% Inhibition of defaecation
$MgSO_4$ (Control)	2 gm/kg	4±0.4082	-
Loperamide	3 mg/kg	1±0.4082 ^{a**}	75%
Bixa orellana extract	250 mg/kg	1.75±0.2500 ^{a**}	56.25%
Bixa orellana extract	500 mg/kg	1.25±0.2500 ^{a**}	68.75%

The values were analyzed by One way ANOVA, Dunnett test: Comapring all vs. control. Values were represented as Mean± S.E.M. The results were considered as statistically significant , more significant and extremely significant when $p<0.05$ *, $p< 0.01$**, $p<0.001$*** . a=compared with $MgSO_4$ control group.

Bixa orellana showed very significant antidiarrheal activity compared to control group both in castor oil and magnesium sulphate induced method. However further bioassay guided pharmacological study is required to identify the exact mechanism of action.

5.Conclusion:

The results of the present investigation indicated that the hydromethanolic extract of Bixa Orellana seeds exhibits promising antidiarrheal properties.Although the precise method by which the crude extract's antidiarrheal activities are exerted remains unknown, it is thought that the antisecretory and antimotility activities present in the seed extract may be responsible for its therapeutic effects.The presence of active phytochemical components, such as flavonoids, tannins, terpenoids, saponins, phenols, and alkaloids, may account for the aforementioned antidiarrheal properties.Hence, the current study justifies the use of Bixa Orellana seeds to alleviate diarrhea in Tropical traditional medicine.

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Ethical Approval:

All animal research was taken place in compliance with the norms for the use of laboratory animals, and the IAEC (Institutional Animal Ethics Committee) of Primeasia University, Dhaka has approved the experimental protocols.

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